DEVELOPMENT OF THE PRIMARY GONAD AND SEXUAL PHASES IN VENUS MERCENARIA LINNÆUS[1]

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Introduction

Sexuality of Pelecypoda has been a subject of scientific study since the end of the seventeenth century. A review of the literature on this subject reveals that in some of the forms that have been carefully studied many individuals exhibit at least one change of functional sexuality during their lifetime (Roughley, 1933; Coe, 1936*a*, 1936*b*). As a rule, a strong tendency toward protandry has been observed. Thus far, however, only two genera of Pelecypoda, namely, *Ostrea* and *Teredo,* have been extensively studied. It is true that these studies have contributed greatly to our knowledge of sex ratios and sexual phases in these bivalves, but it also seems possible that a thorough study of a representative species belonging to a genus widely differing in its mode of living from oysters or *Teredo* might furnish additional information leading to a better understanding of the sexual phenomena of mollusks. *Venus mercenaria,* the hard-shelled clam of the Altantic Coast commonly known as "quohog," was selected for this study.

This proved to be a fortunate choice, for the species was found to pass through phases of sexuality somewhat differing from those previously described for any member of the group. Like the teredos and the oysters, the hard-shelled clam shows a marked degree of protandry but there is no evidence of a second change of sexuality. The primary gonad is more or less distinctly bisexual but in nearly all individuals it becomes differentiated into a spermary when the animal is only a few millimeters in length and only a few months of age. The young individual may function as a male at the end of its first summer, retaining this phase of sexuality during the winter and becoming fully functional at the age of about one year. Others first become functional in their second summer.

About half of all the young clams examined retained the male phase

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of sexuality after the discharge of the spermatozoa, while an approximately equal number transformed to females. The sex ratios during the second winter and thereafter are thus equalized.

The writer desires to express his appreciation to Professor W. R. Coe for his advice and criticism throughout the course of this work.

Material and Methods

V. mercenaria is found along the Atlantic Coast from the Gulf of St. Lawrence to the coast of Texas. In the past few decades this species has become established on the Pacific Coast where it was transplanted with the eastern oyster.

The material generally used in this work was collected in Long Island Sound, near Milford, Connecticut. Young clams were obtained at regular intervals from a single clam bed and prepared for microscopic examination. A large number of small clams was also taken from concrete tide-filling tanks with a capacity of several thousand gallons, at the U. S. Fisheries Biological Laboratory in Milford, Connecticut, where environmental conditions, such as temperature and salinity of the water were observed and recorded. In the preparation of the material for study, standard methods of cytology and histology were employed.

In the present study all the observations on living material were checked by histologically prepared material in order to determine accurately the characteristics of the constituent cells of the gonads. The method employed by Spärck (1925), which consisted of boring a small hole through the shell of an animal and removing a piece of the gonad for examination, is not satisfactory, as very misleading conclusions may be reached by assuming the condition of the entire reproductive system from the examination of a single small sample of the gonad. Similarly, the method of Orton (1927), consisting largely of the examination of the living tissue to note the condition of the gonad with regard to the production of sex elements, is also open to criticism because of the impossibility of distinguishing many important details under such conditions.

Age and Size

A review of the literature fails to disclose any important contributions on the subject of sexuality of *V. mercenaria* in general and on the development of the primary gonads in particular. The only work is that of Belding (1912), where he states that the average hard-shelled clam is capable of spawning when in its third summer, for sexual products

could not be found at an earlier age. The sexes were found to be separate, each animal presumably remaining either male or female all its life. He gives no histological data, and it is apparent from his work that his conclusions are based only on macroscopic examination of living clams and upon his observations of their spawning activities.

It was impossible to obtain for this study small clams whose shell-length was less than 4 mm. long. Therefore the smallest animals studied were 4–5 mm. long. These were found at Trumbull Beach, Long Island Sound, during September and October. As the age of studied animals is of importance, several attempts were made to secure clams of a known age. This was accomplished by collecting seed clams and by keeping them under observation. There are two possible conclusions as to the age of small, 5–7 mm. clams, collected for this study during October–December; namely, that they set in the year they were collected, in which case their age would have been only a few months, or that they were about 14–18 months old, setting the preceding year. Belding (1912), studying *V. mercenaria* along the coast of Massachusetts over a period of five years, found that the rate of growth of the clam is largely determined by its environment, and that, as a rule, the growth in any bed is fairly uniform. In his experiments at Monomoy Point, he found that the average size of a 14-months-old clam (collected in October) was 25.59 mm. Judging by the description of the Monomoy Point experiment, the environmental conditions there closely resemble those of Trumbull Beach, at which place the young clams were obtained for the present work. The similarity of conditions of the two places makes it logical to assume that the rate of growth of clams at Trumbull Beach is more or less identical to that of clams of Monomoy Point. Thus, clams 5–7 mm. long, collected in October–December, presumably were of that, and not of the preceding year set. This conclusion is supported by the observation of Belding (1912) that young clams immediately after setting showed an average gain of 3.4 mm. per month, which would make a length of about 10 mm. by the end of November.

In the present paper all the remarks concerning the size of animals refer to the length of the shell.

Development of Primary Bisexual Gonad

Gonad tissue first appearing in the young animal consists of a very thin layer of cells between the muscular body wall and the stomach. Gonads of the juvenile phase first appear, not near the muscular body walls, but a short distance from them at the level of the heart or slightly below it. The follicles of the juvenile gonad are at first composed of a single layer of germinal epithelium cells. These cells of elongated type

are rather irregular in shape and size and possess very large, deeply-staining nuclei. During the early stages there is virtually no lumen

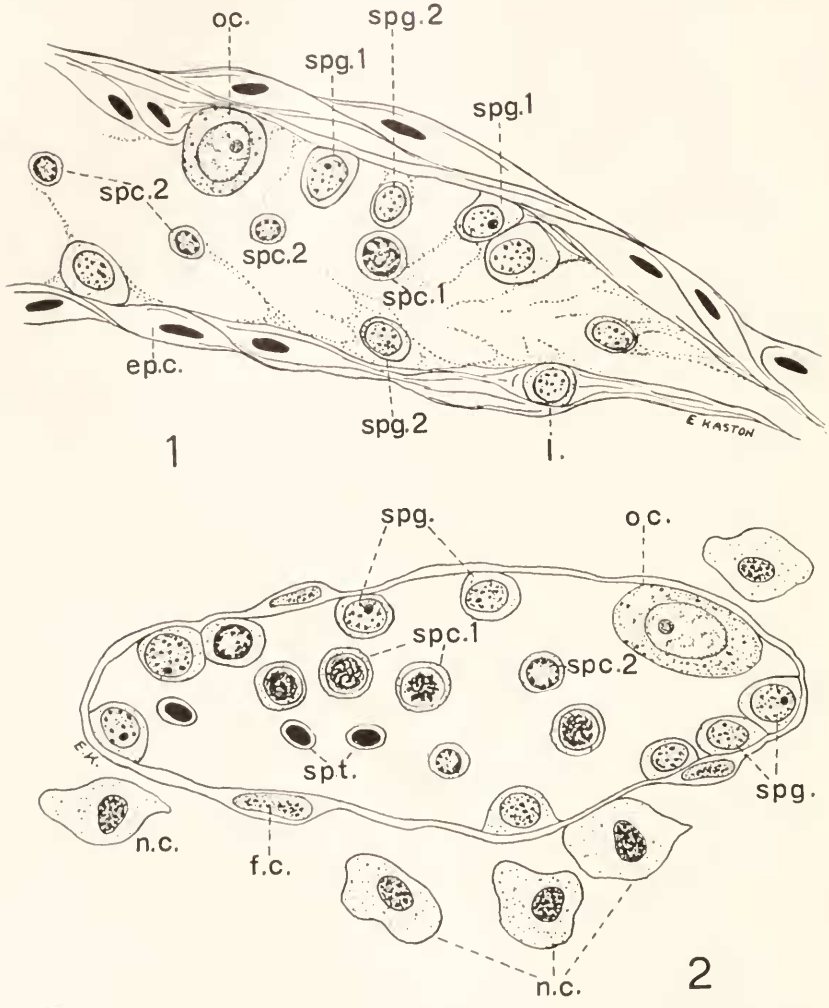


FIG. 1. Primitive bisexual gonad of a young clam soon after formation of the lumen. *l*, indifferent cell; *ep.c.*, follicular cells; *oc.*, young ovocyte; *spg.1*, primary spermatogonia; *spg.2*, secondary spermatogonia; *spc.1*, primary spermatocytes; *spc.2*, secondary spermatocytes.

FIG. 2. Primary bisexual gonad in a young clam (4-6 mm.) showing large ovocyte (*oc.*), spermatogonia (*spg.*), primary spermatocytes (*spc.1*), secondary spermatocytes (*spc.2*), and spermatids (*spt.*); *f.c.*, follicular cells. Nutritive cells (*n.c.*) are seen near the follicular wall.

formed, the walls of the follicles being almost in contact with each other. As the animal grows, the follicles of the primary gonad begin

to ramify through the loose connective tissue. A few weeks later the germinal epithelium begins to differentiate into gonia and at this time the lumen in the follicle is formed (Fig. 1). Rapid proliferation and specialization of cells follows.

In small animals, 5–12 mm. long, the gonad does not extend very far in the ventral direction, seldom reaching below the line corresponding to the middle part of the stomach. The follicles of the gonad are not very numerous, and usually only six to eight of them can be found in one cross-section of the entire animal. In cross-sections the gonad follicles of such animals already show the lumen, which is usually oval or round in shape with a greatest diameter of 50–100 microns. Careful study of sections reveals that different gonad follicles of the same animal exhibit a widely different degree of development and bisexuality. Some of the follicles may consist of only a few indifferent cells while in others the germinal epithelium has already differentiated into male and female cells. All degrees of such processes can be observed. In some cases the follicle contains a few gonia along its wall and a mass of spermatogenic cells in early stages of development in the lumen (Fig. 2). In other, more advanced, cases spermatozoa are already formed, sometimes occupying the largest part of the lumen, while a few small ovocytes showing mitochondrial filaments of yolk nuclei are found along the walls of the follicle (Fig. 3). Gonads at such stages are distinctly bisexual and because the proliferation of spermatogenic cells is very rapid such young gonads acquire a strong male appearance (Loosanoff, 1936*a*).

In some cases the appearance of the follicles looks as though the spermatozoa were already discharged. For instance, a large sample of animals 5–7 mm. long was collected on Trumbull Beach in October, 1934. Upon examination of the prepared material it was observed that some of the follicles had already discharged spermatozoa. The lumen of such follicles was large and empty while along its walls young ovocytes and indifferent cells were present. As a rule, few phagocytes could be found inside the lumen (Fig. 4). With the purpose in view of establishing the age of those animals, very careful examination of their shells was made under a dissecting microscope. In not a single case was an indication of the winter ring found. The outer surfaces showed an embryonic shell and 7–13 growth lines formed at approximately equal intervals (Fig. 5). Thus the studies of the shells indicated that the animals of that sample were only a few months old.

If the evidence that *V. mercenaria* produces and discharges gametes during the first summer of its life is reliable, then it resembles in this respect many of the other bivalves whose sexual development is well

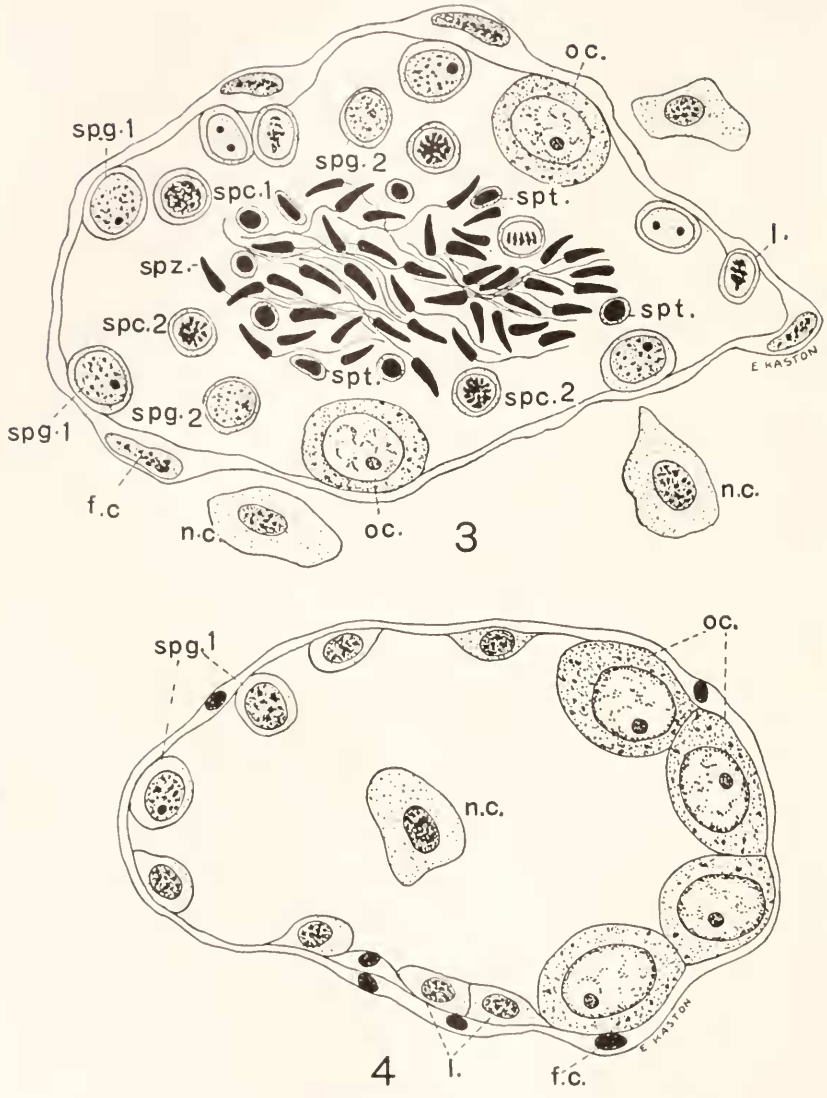


FIG. 3. Primary bisexual gonad of predominantly male character. Spermatozoa are present, occupying the center of the lumen. A few ovocytes (*oc.*) are lying along the follicular wall. *l*, indifferent cells; *spg.1*, primary spermatogonia; *spg.2*, secondary spermatogonia; *spc.1*, primary spermatocytes; *spc.2*, secondary spermatocytes; *spt.*, spermatids in different stages of spermioteleosis; *spz.*, spermatozoa; *n.c.*, nutritive cells; *f.c.*, follicle cells. Size 7–12 mm.

FIG. 4. Gonad follicle of a young clam (7 mm.) after discharge of the first crop of spermatozoa. *l*, indifferent cells; *oc.*, young ovocytes; *spg.1*, primary spermatogonia; *n.c.*, phagocyte; *f.c.*, follicle cells.

known (Nelson, 1922; Coe, 1936*c*). It is apparent, however, that there cannot be a definite rule applied to all Pelecypoda mollusks as to the time when they first form functional gametes. As a matter of fact, considerable differences are often found in closely related species and in different localities, as Spärck (1925) has so clearly demonstrated in *O. edulis*. *O. lurida* is capable of passing through three sexual phases during the first year of development in warm waters (Coe, 1932), while *O. virginica* of the northern part of the eastern coast of the United States does not form any ripe gametes until the second year (Needler, 1932). In *V. mercenaria*, ripe spermatozoa are formed within 3–5 months after setting. Among several hundred young animals studied, approximately 98 per cent of them passed through such a protandric male phase. The remaining 2 per cent appeared to develop into females without passing through a functional male phase.

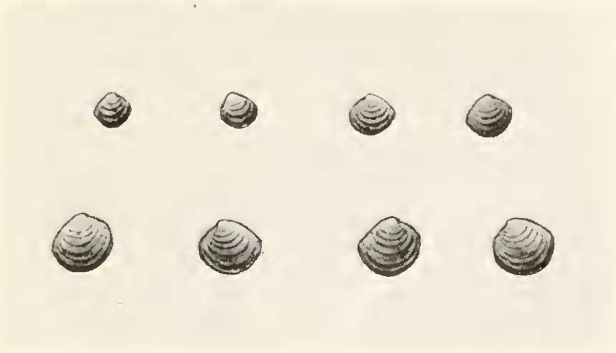


FIG. 5. Shells of the young clams, age about 3 to 4 months, collected in October–November, showing the absence of the winter ring. All of these were already provided with gonads containing mature spermatozoa. Natural size.

CONDITION DURING FIRST WINTER

According to the writer's observations, the shell-growth of clams in Long Island Sound ceases in November. Small (6–10 mm.) clams examined at that time differ very little from those studied in October. Again gonad follicles in various stages of development are found. In many follicles spermatogenesis continues and cells of all stages of development, including spermatozoa, are present (Fig. 6). In other cases spermatozoa have already been discharged and the distended lumen is virtually empty (Fig. 4). Often numerous phagocytes are seen invading the lumen or attached to the walls of the follicle. Those presumably ingest the residual spermatic cells. In a few instances the gonad tissue has already ramified ventrally below the stomach and individual follicles

can be seen confined to the space between the muscular body wall and the digestive gland.

By the middle part of December the temperature of the water of Long Island Sound reaches the point at which the period of hibernation for this species begins (Loosanoff, 1936*b*). Examination of young animals collected during that period, which extends until the middle part of April, or until the water temperature rises above 5.0° C., reveals that

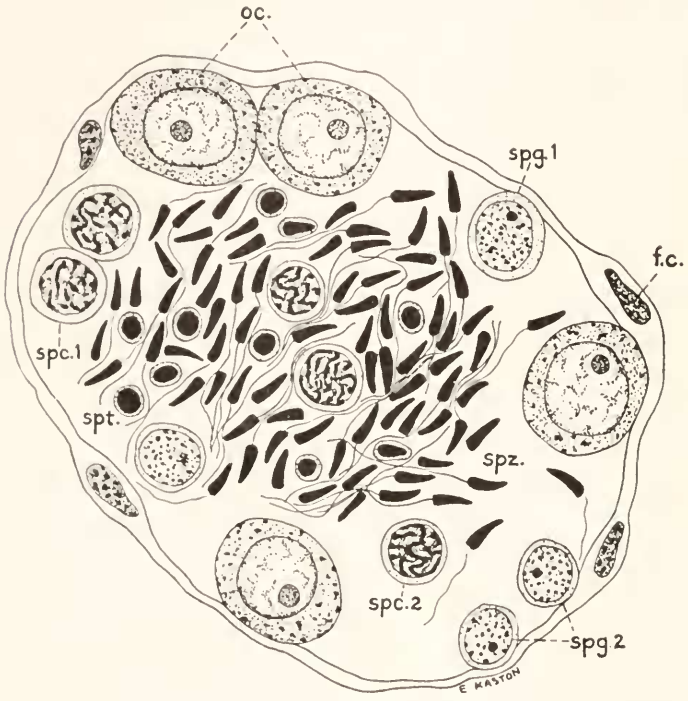


FIG. 6. Bisexual primary gonad of a young individual about 16 weeks of age showing the cells of both sexes but predominantly of male type. *spg.1*, primary spermatogonia; *spg.2*, secondary spermatogonia; *spc.1*, primary spermatocytes; *spc.2*, secondary spermatocytes; *spt.*, spermatids; *spz.*, spermatozoa; *oc.*, large ovocytes showing mitochondrial bodies and yolk nuclei; *f.c.*, follicle cells.

changes of only minor importance occur in the primary gonads. There is apparent neither increase in the number of cells constituting the gonad, nor increase in the size of the follicles. Spermatogenesis is virtually discontinued and cells of the intermediate stages are few in number. The spermatozoa, however, are retained in large numbers in many of the follicles. Unless carefully examined, many of the animals at this stage may be mistaken for true males, but the presence of young ovocytes showing distinct mitochondrial bodies and yolk nuclei indicates the actual

bisexual character of the gonads. In other cases the bisexuality of an individual is more easily noticed because of the large number of young ovocytes present (Fig. 6). A few pycnotic cells are sometimes seen.

Hibernation continues until the middle of April. The lowest temperature of the year is reached in February or the first part of March. After the middle of March the water temperature begins to rise very slowly and reaches 5.0° C. by the middle of April.

Condition in Spring and Summer

By the time the water temperature reaches 7.0–8.0° C., which usually takes place at the end of April, some minor changes in the gonads of young animals begin to occur. They are manifested by resumption of spermatogenesis in some of the individuals. The process, at that time, is very slow, and newly-formed cells, mostly spermatocytes of the first and second order, are few in number. The majority of animals still have gonads in the state resembling that of the hibernation period. As soon as the water temperature advances to a 10° C. mark, usually by the middle of May, more pronounced changes take place in the gonads. Active spermatogenesis is resumed in the follicles of many animals. Numerous gonia, primary and secondary spermatocytes are formed but few spermatids are seen. In many follicles spermatozoa retained since the preceding autumn are present. At this time a slight extension of the follicle is already noted. Phagocytic-nutritive cells begin to surround the follicles in which gametogenic activities are going on. Gonads continue to retain their bisexual nature because many small ovocytes measuring 20–30 microns can always be found in all follicles, even those showing a distinct preponderance of spermatogenic cells. Young ovocytes are, as a rule, found lying in contact with the follicular wall. Their large nuclei make them easily distinguishable from other cells.

Phagocytic-nutritive cells are often found in large numbers along the outer walls of the gonad follicles and a few penetrate into their lumens (Fig. 7). These cells, measuring up to about 12–14 microns and having a nucleus 4–4.5 microns in diameter, occur as a rule near the follicular wall. Their numbers appear to vary with the seasons, the greatest number being found during the active stages of gametogenesis. They are found during all stages of development from indifferent gonad to mature stages. Often some of them project through the follicular walls into the lumen, while others invade it. In clams such cells appear to perform functions of two types. First, their intimate contact with the gonad during the most active stages of gametogenesis indicates that they contribute certain substances necessary for the developing cells. Thus, their nourishing function may be assumed. Second, their pres-

ence inside the gonad follicles immediately after spawning suggests their purely phagocytic rôle of removing partially cytolysed, degenerated and residual cells.

As the season advances and the water temperature gradually increases, reaching about 15.0° C. by the end of June, rapid proliferation of sex cells progresses in a parallel manner. Gonads of young animals examined at that time of the year show that spermatogenesis proceeds very actively and that spermatogenetic cells in all stages of development are filling the follicles, often occupying the entire space of the lumen. It often happens that various follicles of the same animal

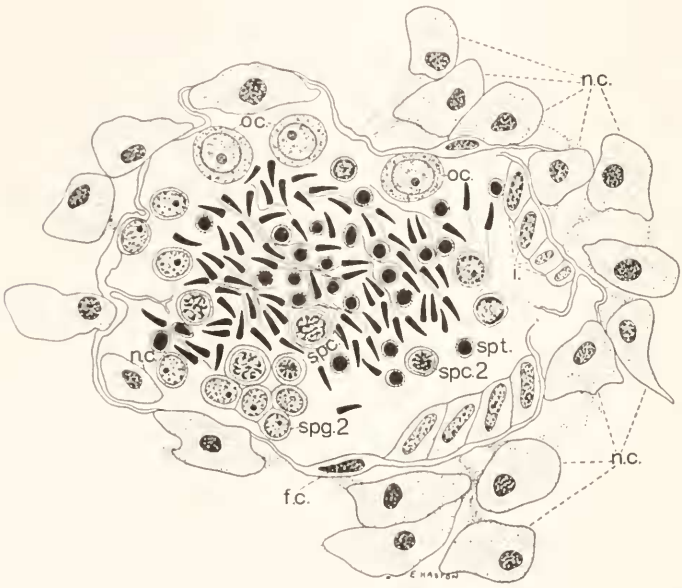


Fig. 7. Gonad of the young clam (1.0 cm.) surrounded by the nutritive phagocytic cells (*n.c.*). Other letters as in Fig. 6. Late April–May.

show quite different stages of development. Frequently one of the follicles possesses a large number of spermatozoa, while another follicle, situated next to it, has most of its cells in early stages of spermatogenesis. Nutritive cells are quite numerous, sometimes entirely surrounding the follicle (Fig. 7). The clams are feeding and growing rapidly, many of them showing an increase in total length of shell of 3 mm. since the end of the hibernation period.

On examining the clams passing through active gametogenesis, one's attention is immediately drawn to the large numbers of nutritive cells surrounding the outer walls of the follicles. In the follicles themselves

great activities are noticed. They are quite distended and filled with spermatogenetic cells. At this time mature spermatozoa are already more numerous than cells of other stages. Spermatocytes in all stages of development and spermatids in spermioteleosis are found. Regardless of such spermatogenetic activities and the distinctly male character of gonads, young ovocytes are always found in small numbers along the wall of the follicles. There is a rapid extension of the branching system of follicles, but this extension is directed chiefly in the posterior direction and not ventrally; consequently very few follicles extend below the level of the stomach.

As has been mentioned before, some of the animals examined during the winter had follicles virtually devoid of all except indifferent cells. Such animals apparently discharged their spermatozoa in the fall. In June and July those animals also exhibit gametogenetic activities and spermatozoa are rapidly formed. It is very interesting to note, however, that in such cases the follicles usually contain more female cells than the follicles of the animals which retained spermatozoa throughout the winter. The variation in the proportion of cells characteristic of the opposite sexes found in clams of the same age strongly indicates that genetic factors and probably the effect of environment influence the production of sex cells.

Spawning

In August the water over the natural clam beds reaches a temperature high enough to induce the spawning of clams. If young clams, which by this time have reached the size of 2.0–3.0 cm. are examined, their gonads reveal the fact that the spawning process is not a phenomenon of short duration but lasts for some time. Different follicles of the gonad of the same animal contain sex cells in various quantities. Some follicles have already discharged their contents while in other follicles spermatogenesis is in progress and the lumen is still filled with mature spermatozoa. Apparently, several days, or perhaps weeks, are required for young clams to complete their spawning. Furthermore, by examining a sample consisting of many animals, the conclusion can be reached that there is considerable difference in the spawning behavior of individuals because some of the animals collected at the same time and from the same place have their sex products completely discharged, while others still retain their spawn in various quantities. This indicates that the entire population of a certain bed does not begin spawning at exactly the same time, and that the spawning season of clams extends for a considerable period of time.

In many follicles, which during the spawning period are partly or

fully freed of spermatogenic cells, young ovocytes, many of them in the spireme stage, are growing along the walls. In some instances groups of ovocytes occupy considerable portions of follicles. A few nutritive cells may be observed in the vicinity of follicles whose contents are already discharged. Branching gonad tubules begin at this time to extend ventrally, occupying the space between the body walls and the digestive gland.

During the months of August and September the animals grow very rapidly, reaching the size of 2.5 to 3.2 cm. Many animals have grown as much as 2.0 cm. since the end of the hibernation period. Simultaneously with the growth of the animals, ramification of gonad follicles proceeds. The follicles spread in all directions and envelop the stomach and intestines as well as the spaces in connective tissue between the body wall and the digestive gland.

Transformation to Definitive Males and Females

In September, after the spermatozoa have been discharged, two types of individuals become distinguishable as definitive males and females. In the males a second or third period of spermatogenesis begins in the autumn and continues at a reduced rate throughout the winter. Mature spermatozoa are retained in the follicles all this time. Many young ovocytes and numerous indifferent cells are also present. In the spring, with the increase of water temperature, renewed rapid branching of follicles takes place and the gonads then begin to acquire the typical male character of the adult. Spawning follows later in the summer.

In animals destined to become females radical changes take place in the gonads upon the completion of the initial male phase. After the discharge of spermatozoa the follicles are left in a distended state. Their lumens are large and empty (Fig. 8) with the exception of a few pycnotic bodies consisting of cytolysed spermatogenic cells. An irregular layer of indifferent cells, ovogonia and primitive ovocytes measuring up to 20–25 microns, and a few spermatogonia remain for a time along the walls of the follicles. Nutritive cells lie in close contact with the gonad walls and occasionally can be found inside the lumens of the follicles. No significant changes occur in the gonads of future females during the hibernation period, for during all this time the follicles remain distended, round or oval in outline, with virtually empty lumens. There is little indication of ovogenetic activities. In the middle of May, regardless of the fact that the water temperature is 9.7° C. (i.e., about four degrees above hibernation mark), and that the animals already had been feeding for approximately one month, their gonads remain in a semidormant

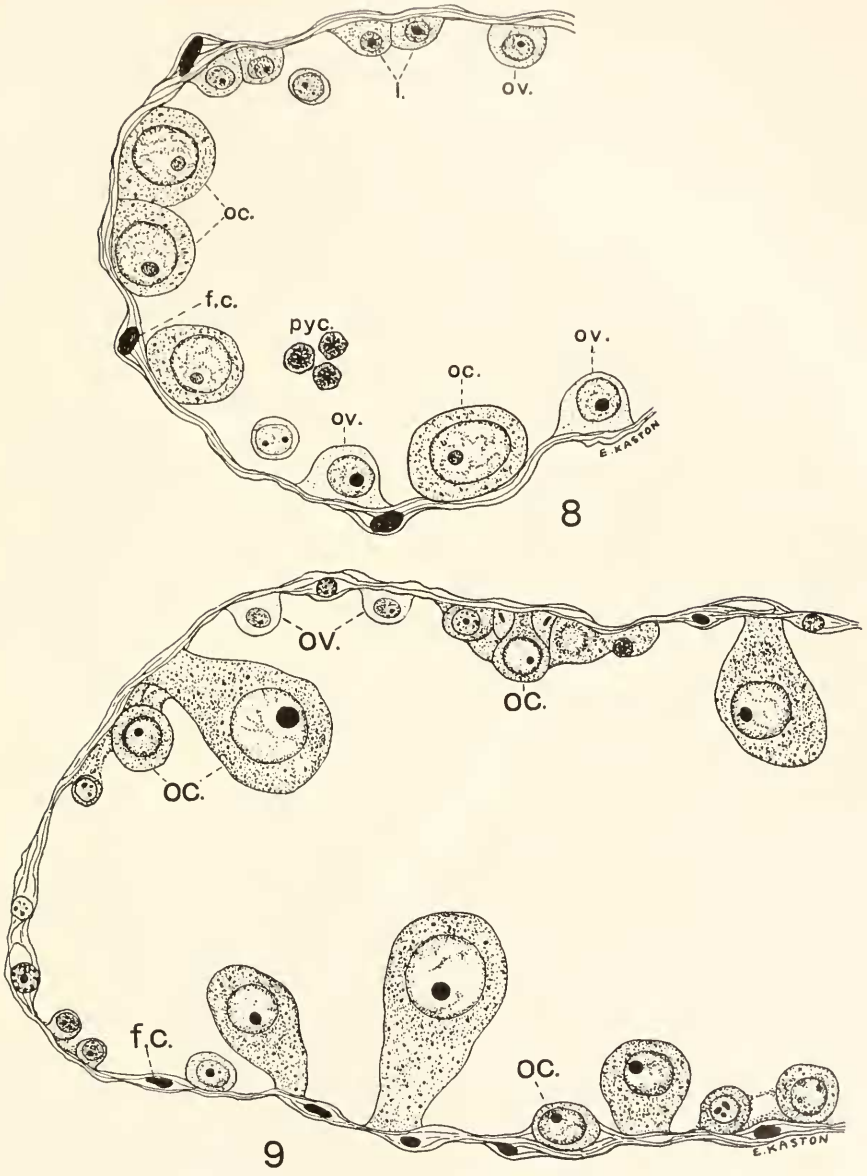


Fig. 8. The gonad of the animal destined to become a female after the completion of the initial male phase. *I*, indifferent cells; *ov.*, ovogonia; *oc.*, young ovocytes; *pyc.*, pycnotic cells; *f.c.*, follicle cells.

Fig. 9. Early ovogenetic activities in the young female clam producing its first crop of eggs; *ov.*, ovogonia; *oc.*, ovocytes in various stages of development; *f.c.*, follicle cells. Age 18–19 months. Middle of May.

state. Only in rare instances are synaptic activities in young ovocytes noticed. In some follicles, however, pycnosis and phagocytosis of spermatogenetic cells left over from the last autumn proceed very rapidly.

In clams producing their first crop of ova, slow ovogenetic activities become apparent in the middle of May and active ovogenesis begins in June when the water temperature approaches 15° C. Within a few days after such a temperature has been reached the appearance of the gonads undergoes a marked change. Young ova in various stages of development, some of them 42–45 microns in size, grow from the walls of the follicles into the lumen (Fig. 9). The growth and proliferation of ova are very rapid and the animals formerly functioning as males have now reached the stage of functional females. Not all the follicles begin to produce ova at the same time. Frequently, in the same animal some of the follicles contain large, rapidly growing ova, while a few others are still in an apparently dormant state showing many indifferent cells along their walls, ovogonia and very minute ovocytes.

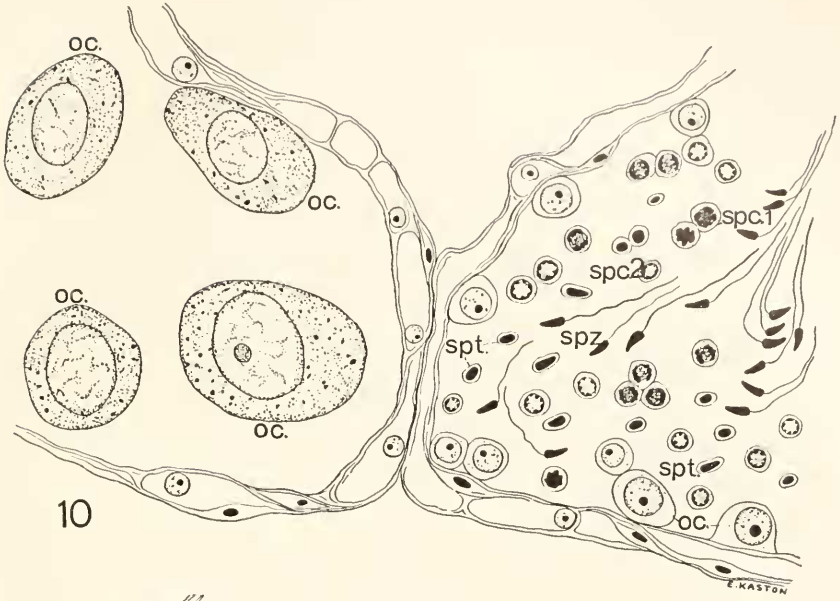
The first crop of eggs produced by the young female is usually small and in this respect they can always be distinguished from fully adult individuals, because in the latter case the follicles contain many more ova. After spawning, the gonads of both sexes may contain some cells characteristic of the opposite sex, thus retaining their bisexual character. It is especially noticeable in the case of males, where comparatively large ovocytes are easily distinguished from male cells.

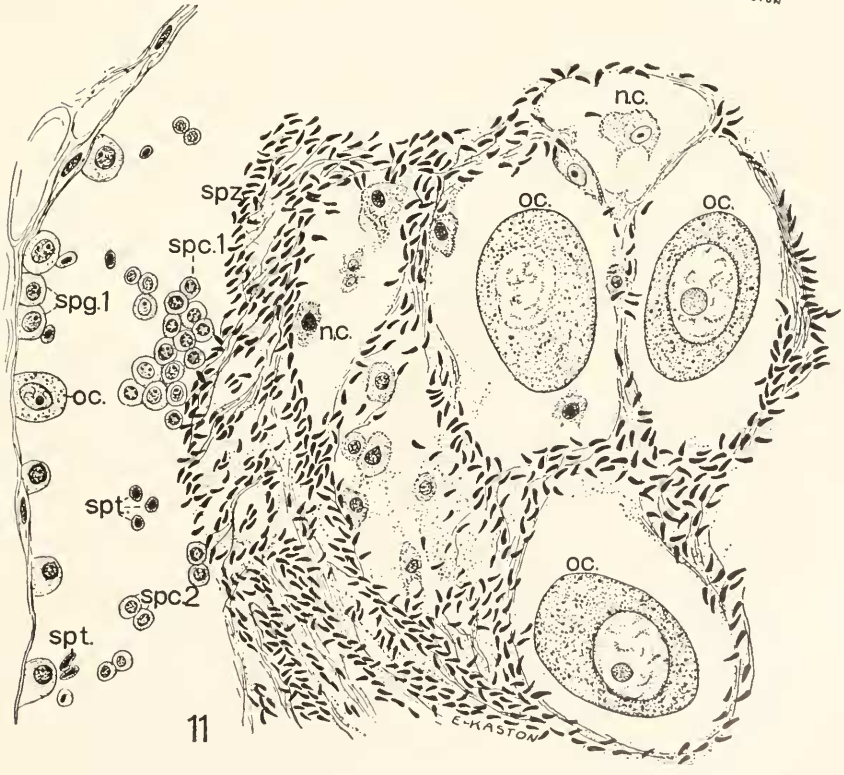
Functional Hermaphroditism

Pelseneer (1926) has shown that hermaphroditism is quite common among mollusks. Among Pelecypoda numerous cases of partial and true hermaphroditism have been reported. As has been stated in the chapter on the development of the primary gonads of *V. mercenaria*, many grades of bisexuality occur in that animal during the first two years of its life. The examination of the gonads of adult males also reveals, in almost every instance, the presence of small ovocytes somewhere along the walls of the follicles. In all such cases, since the cells of both sexes are present in the gonads of the same individual, the term "partial hermaphroditism" may be applied. However, under functional or true

Fig. 10. Functional hermaphroditism in *Venus*. Portions of two adjacent follicles of the same individual containing cells of opposite sexes. *spc.1*, primary spermatocytes; *spc.2*, secondary spermatocytes; *spt.*, spermatids; *spz.*, spermatozoa; *oc.*, large ovocytes showing mitochondrial bodies and yolk nuclei.

Fig. 11. Portion of hermaphroditic gonad showing the ripe ova surrounded by spermatogenetic cells. *spg.1*, primary spermatogonia; *spc.1*, primary spermatocytes; *spc.2*, secondary spermatocytes; *spt.*, spermatids; *spz.*, spermatozoa; *oc.*, large ovocytes showing mitochondrial bodies and yolk nuclei.





FIGURES 10 AND 11

hermaphrodites, only those animals should be included whose gonads possess masses of cells of both sexes, in which case self-fertilization may be possible.

In *V. mercenaria* the adults are, with few exceptions, of separate sexes. Among 650 mature clams studied by means of serial section, only 3 cases of functional or true hermaphroditism were observed. In one case fully ripe ova were found in some of the follicles, while spermatozoa occupied an adjacent but separate portion of the gonads (Fig. 10). In two other cases a few ripe ova were occupying the lumen of the follicle in which spermatogenetic activities were also almost completed. Numerous ripe spermatozoa surrounded the ova lying in the follicles (Fig. 11). In all of these instances the ova and spermatozoa may be discharged simultaneously into the mantle cavity of the animal and then into the water where abundant opportunity for self-fertilization offers itself.

Summary

1. Examination of the developing gonads of young clams from Long Island Sound, at two-week intervals during the first year of life, shows that a primary bisexual gonad is formed in each individual when the animal reaches the size of 4–6 mm.

2. The primary gonads form from associations of germinal cells in the connective tissue, not immediately beneath the body walls but at some distance from them, at the level of the heart or slightly below it. A lumen soon appears in each gonad follicle and the germinal cells begin to proliferate rapidly.

3. The primary gonad contains the antecedent cells of both sexes but the protandric nature of the primary gonad becomes manifested by the rapid proliferation of the spermatogenic cells.

4. This species is not strictly protandric because few individuals develop into females without passing through a functional male phase.

5. Functional spermatozoa have been observed in the gonads of young clams, collected in October and November when only 5–7 mm. long. In some cases discharge of spermatozoa occurs at that time.

6. In September of the second year, after the animals have spawned as males, two types of individuals become distinguishable as definitive males and females. In the males a second period of spermatogenesis begins in the autumn and continues at a reduced rate throughout the winter. The bisexual character of the definite sexual gland is usually retained to at least some extent because of the presence of many small ovocytes.

7. In animals destined to become females, after the completion of the initial male phase, the gonads may remain virtually empty but in a distended state throughout the winter. There is little indication of ovogenic activities until the following spring when the water temperature approaches 15° C. From then on the proliferation and growth of ova proceeds very rapidly until the animal reaches the functional female phase.

8. In *V. mercenaria* the adults are, with few exceptions, of separate sexes. Among six hundred and fifty mature clams studied by means of serial sections only three cases of true hermaphroditism were observed.

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